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Plasma irradiation of artificial cell membrane system at solid–liquid interface

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We provide direct evidence of plasma-induced pore formation in a cell membrane model system. We irradiated plasma on the basis of the dielectric barrier discharge onto a supported lipid bilayer (SLB). Observation with a fluorescence microscope and atomic force microscope revealed the formation of pores on the order of 10 nm–1 μm in size. Capturing these micropores in a fluid lipid membrane is a significant advantage of the SLB system, and quantitative analysis of the pores was performed. Stimulation with equilibrium chemicals (HNO_3 and H_2O_2) indicated that other transient active species play critical roles during the poration in the SLB. © 2014 The Japan Society of Applied Physics

Atmospheric plasma is applied as a novel and valuable tool in the medical and biological fields.[1–3] Recent studies have demonstrated that the plasma irradiation of cells or organs is applicable multifariously, including sterilization,[4–8] selective killing of tumor cells,[9] cellular regulation,[10,11] and wound healing.[12] In spite of the wide variety of medical and biological applications of plasma, many aspects remain unclear, including what parts of plasma affect cells and tissues and how they do so. Atmospheric plasma generates various active species called reactive oxygen and nitrogen species (ROSs and RNSs) including acidic and radical species,[6–8] which transfer to cells through the medium around the cells. The critical effect of these active species on cells may be a direct effect on cell surfaces or an indirect one through physiological cascades inside cells and/or genetic damages. Biological cell membranes, also called plasma membranes, act as barriers to maintain the integrity of cells by separating the inside from the outside. Chemically active species produced by plasma irradiation first access the cell membranes. Some recent studies showed that cell membranes act as a barrier against plasma irradiation for genomic damage or oxidative protein modification,[13,14] while other studies showed that plasma irradiation causes the oxidation of the intracellular organelles because of the permeation of ROS through the cell wall and cell membrane, without a major deformation of the membranes.[6,15] How the plasma-induced active species affect and/or permeate cell membranes is unclear. Physical damage to a cell membrane, such as poration, is effective for the transportation of solutes including plasma-induced active species into cells, but such a non-selective leakage itself is highly toxic for cells, similar to pore-forming proteins.[16] Permeation via dissolution into the hydrophobic core of a cell membrane is inefficient for the water-soluble active species and possibly prevented by drug efflux pumps.[17] The lipid peroxidation induced by physiological ROSs is also related to cell damage and various pathological states.[18–20] A fundamental understanding of the effects of plasma on biomolecules and their assembled structures is demanded for the further progress and establishment of safety in the medical and biological application of plasma.

Artificial lipid bilayers are useful biomembrane model systems for investigating the fundamental interaction between cell membranes and medical and biological agents.[21–25] The artificial planar lipid bilayers formed at solid–liquid interfaces are called supported lipid bilayers (SLBs), and the spherical lipid bilayers dispersed in aqueous solutions are called lipid vesicles. SLBs have the advantages that fluid and fragile lipid membranes exist stably owing to the support of the solid substrate and that high-resolution surface scientific techniques such as atomic force microscopy (AFM) are adopted. Recently, Svarnas et al. reported the plasma irradiation of a suspension of multi-lamellar vesicles (MLVs),[26] which is the only example of plasma irradiation of an artificial lipid bilayer, to our knowledge. The plasma irradiation induces the fusion of MLVs, but the leakage of the encapsulated solution in MLVs is limited.[26]

In this study, we investigated the effect of the atmospheric plasma on the basis of the dielectric barrier discharge (DBD) on an artificial cell membrane using the SLB system. We visualized that the irradiation of the DBD-plasma to an SLB of phospholipid-generated pores with a diameter on the order of 10 nm–1 μm. The pore formation proceeded without a change in pH, a comparison with the effects of the chemicals in the equilibrium condition indicated the critical roles of the plasma-induced transient active species.

A schematic of the DBD-plasma irradiator developed for this study is shown in Fig. 1(a). A quartz plate was fixed on each of the upper and lower electrodes, which were used to apply an AC voltage. A sinkhole was fabricated on the lower quartz plate and used as a dish for the SLB formation, DBD-plasma irradiation, and observation on the SiO_2/Si substrate. The distance between the upper and lower quartz plates was 1.5 mm. The thickness of the buffer solution above the SiO_2/Si substrate was ~0.47 mm, and the solution was carefully treated so as not to expose the SiO_2/Si surface to the air, because the SLB stably exists only in an aqueous solution. We settled the DBD-plasma irradiator in a glove box and purged its inside with Ar for 5 min before applying an AC voltage of 15 kV and 15 kHz between the electrodes to generate the DBD-plasma. The plasma was irradiated to the SLB on the SiO_2/Si substrate for 30–150 s through the buffer solution. The increase in the temperature due to the plasma irradiation was suppressed by the circulated coolant in the electrodes. The DBD in the Ar gas ambient, which probably included a slight amount of residual air, contained rapidly moving bright filamentary discharges [Fig. 1(b)]. The details of the instrument are described elsewhere.[27,28]

We used dioleoylphosphatidylcholine (DOPC) because phosphatidylcholine is the representative phospholipid most abundantly existing in cell membranes. A vacuum-dried film of DOPC mixed with a fluorescence-labeled lipid [dioleoyl-